# Gold Nanoparticles Treatment in MCF-7 Spheroid Co-Cultures With Dendritic Cells

Babensee Laboratory for Immunoengineering
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Georgia Institute of Technology

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April 16, 2019

Faculty Member 1:
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# INTRODUCTION

In the last decade biomaterials have increasingly been studied as the next generation of immunotherapeutic treatments for their tendency to induce host response against themselves when implanted. Addressing this significant barrier of host response is a key step in engineering our immune response to target foreign invaders and diseases such as cancer. A promising application of this is gold nanoparticle's (AuNPs) ability to interact with dendritic cells (DCs) in our bodies.

When the immune system responds to a threat in the body, it initiates a twofold response. The innate and adaptive immune responses respond with different approaches to establish immunity in the body, but ultimately work in partnership with each other to optimize the response pathway. While the innate response is immediate and nonspecific to the antigens of importance, the adaptive immune response takes several weeks to developed, but provides a tailored response to the antigens present. The adaptive immune response functions through T and B lymphocytes; allowing the body to retain memory in the form of a chemical structure copy of the antigen of importance. This memory allows the body to later elicit a quicker response to foreign invader upon subsequent exposure. Dendritic cells of the immune system are responsible for mediating both of these reaction pathways.[1]

AuNPs show high potential in the field of immunotherapies because they act as an adjuvant to evoke immuno-suppressive or immuno-activated response depending on their geometries and ligand coatings. The role of dendritic cells is to work as a gatekeeper to initiating immune response in our bodies as they are the most potent antigen presenting cell (APC). In previous research, it has been shown that AuNPs can trigger the maturation of immature dendritic cells (iDCs) to activated or tolerogenic phenotypes.[2]

Researchers continue to fine tune nanoparticle technology for personalized immunotherapies and data indicates the efficacy of AuNP in eliciting anti-tumor response and a providing a superior method to apply targeted vaccinations.[3,4] As the potential for clinical application becomes increasingly apparent, we must dive deeper into the pathophysiology of the tumor environment and subsequently question the relevance of these findings.

The host environments' interaction with the tumor is one of the most important factors in cancer modeling.[5] In this study we investigate effectiveness of AuNP application in the presence of tumor microenvironment equip with dendritic cells, macrophages, and fibroblasts. We iteratively preform co-cultures of MCF-7 breast cancer cells with every combination of these cells in order to create a schematic which will assess applicability of AuNP use in breast cancer models. To make this assessment we will run tests to determine cell growth, viability, metastasis, and susceptibility to drugs.

# LITERATURE REVIEW

Dendritic cells are created and cultivated by differentiation in specific bone marrow cells called monocytes.[6] When an immune response occurs in the body, monocytes will cluster at the site of inflammation and systematically develop into immature dendritic cells (iDCs). Their major role in the innate and adaptive pathways is the activation of T cells.[7] Antigens are presented to immature dendritic cells, the iDCs phagocytose the antigens, and present the antigens on their surface. Once the antigens are presented on the surface of these cells the classification changes from immature to mature.[8]

In the last decade, nanoparticles have been a major area of focus for many researches as they show potential to carry and delivery drugs with very specific targeting, due to their low cell

toxicity. Additionally, they show promise in the augmentation of the maturation of dendritic cells. Controlling the phenotype displayed in a mature dendritic cell allows for engineering and manipulation of the body's immune response.[9]

Changes in nanoparticle size, shape, and coating have been shown to majorly skew the effectiveness of such treatments. Treated on a wide range of cell types, gold nanoparticles (AuNPs) in rod shapes with diameters of approximately 55nm seem to be the most effective in cell targeting.[10] This is a good starting point for functionalization of NPs, but studies indicate that they should be further functionalized on a by-cell basis. For example, studies have shown that folic acid (FA) increased AuNP uptake in tumor cells because they over express folate receptors on their surfaces.[11] As we dig into how the functionalization of a NP determines its effectiveness, it becomes clear that the cells' microenvironment is just as important as cell type, if not more important, to determine binding efficiency.

3D extracellular matrices modulate intercellular interactions such as cell growth and metastasis.[12] Additionally, no tumor cell is purely one cell type. Fibroblast, dendritic cells, and monocytes are all part of the mix. Co-cultivation of these cell types into 3D spheroids produce protein concentrations analogous to tumors, while 2D models do not.[13] Studies have actually shown that spheroids are produced are predictably and analogous to one another and to actual tumors when co-cultured with fibroblasts.[14]

MCF-7 breast cancer cells are one of the most commonly used breast cancer cell lines for research, because they react similarly to breast cancer cells in the body and they can be functionalized to be drug resistant to cancer therapies.[15] They are fairly adherent cells and have a karyotype of 69 chromosomes.[16] Spheroid cultivation of MCF-7 cells can be done in a variety of methods include ultra low attachment (ULA) plates and embedment in Matrigel and show better

results than other cell lines.[8] Additionally, these methods allow for easy co-culture of MFC-7 cells.

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AuNPs show high potential in the field of immunotherapies because they act as an adjuvant to evoke immuno-suppressive or immuno-activated response depending on their geometries and ligand coatings. The role of dendritic cells is to work as a gatekeeper to initiating immune response in our bodies as they are the most potent antigen presenting cell (APC). In

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The host environments' interaction with the tumor is one of the most important factors in cancer modeling.[8] In this study we investigate the effectiveness of AuNP application in the presence of tumor microenvironment equipped with dendritic cells, macrophages, and fibroblasts. We iteratively perform co-cultures of MCF-7 breast cancer cells with every combination of these cells in order to create a schematic which will assess applicability of AuNP use in breast cancer models. To make this assessment we will run tests to determine cell growth, viability, metastasis, and susceptibility to drugs. An indication of AuNPs working to combat cancer cells would be decreased cell growth, viability and metastasis and an increased susceptibility to drugs.

# LITERATURE REVIEW

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the perivascular region. This “stockpile” was counterproductive to the deep diffusion of AuNPs into the tumor space because the extracellular matrices of the tumors block NP diffusion in a size dependent manner.[15]

MCF-7 breast cancer cells are one of the most commonly used breast cancer cell lines for research, because they react similarly to breast cancer cells in the body and they can be functionalized to be drug resistant to cancer therapies.[16] They are fairly large adherent cells and have a karyotype of 69 chromosomes.[17] MCF-7 cells have the ability to process estrogen, therefore they are estrogen sensitive. The typical cell size of an MCF-7 cell is 20-25 microns and typically are cultured in Eagle’s Minimum Essential Medium (EMEM). When grown *in vitro*, the epithelial like cells of the culture will grow in monolayers while other cells can produce dome-like shapes. Spheroid cultivation of MCF-7 cells can be done in a variety of methods include ultra-low attachment (ULA) plates and embedment in Matrigel and show better results than other cell lines.[8] Additionally, these methods allow for easy co-culture of MFC-7 cells.

MCF-7 cells are often manipulated to be drug-sensitive to doxorubicin, a chemotherapy drug, or doxorubicin-resistant.[18] Many studies investigate these drug-differentiated phenotypes of MCF-7 cells with their surroundings; such as the study performed by Professor Mougel’s team in France- it investigated the effects on immune reactivity of MCF-7 cells by monocytes. The study found that, according the phenotype of the breast cancer spheroid, monocytes respond with differential recruitment and behavior towards the masses.[19] The type of monocyte that is of particular interest of those in the immune-reactivity field is a macrophage cell. Recent literature suggests that in tumor environments there is an important “cross-talk” that is occurring between breast cancer cells and macrophages. Donzelli noted the increase in angiogenesis as a result of signaling proteins being exchanged between the two cell types.[20] The other major constituent of

breast cancer stroma is fibroblasts. Research indicates that both fibroblasts and macrophages cooperate to organize co-cultures into tumor spheroids *in vitro.* Rama-Esesndagli's group found that not only was there a higher frequency of spheroids formed when using co-cultures rather than just breast cancer cells, but these spheroids also exhibited a higher cellular density than their counterparts and are more analogous to each other.[14]

# MATERIALS AND METHODS

This investigation is multifaceted in terms of experimental setup. First, the proper Matrigel concentration for the formation of MCF-7 spheroids must be confirmed. MCF-7 Cells will be plated at varying cell concentrations in mediums with varying composition of Matrigel to investigate which method most reliably produces spheroids. See the below plate layout for more information.

| | 1% Matrigel to equal parts colagen I | | | 3.5% Matrigel | | | 3.5% Matrigel to equal parts collagen I | | | control: just media | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | cell seed count |
| A | | | | | | | | | | | | | 5,000 |
| B | | | | | | | | | | | | | |
| C | | | | | | | | | | | | | 10,000 |
| D | | | | | | | | | | | | | |
| E | | | | | | | | | | | | | 15,000 |
| F | | | | | | | | | | | | | |
| G | | | | | | | | | | | | | |
| H | | | | | | | | | | | | | |

*Figure 1: Plate layout for determining optimal Matrigel concentration*

Cell viability will be assessed both optically (through the use of a microscope with the capability to take photographs and by using a Cell-Titer Glo 3D Cell Viability assay. The Cell-Titer Glo 3D Cell Viability assay will be performed as instructed by Promega, the distributor.

The next experiment to be performed will be adding AuNP treated dendritic cells to the MCF-7 cells in Matrigel to create a coculture of the two. Dendritic cells will be treated with AuNPs per the protocol perfected by another group of undergraduates in Dr. Babensee's lab. (their paper will eventually be linked here, but it has not been published yet). Subsequently,

macrophages will be added to the coculture and then fibroblasts native to human breast tissue. Assays will be conducted to determine cell growth, viability, metastasis, and susceptibility to drugs under these varying conditions.

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Sherry Sarkar
10:24 AM Sep 20
Resolve

What are the two responses? Innate and adaptive? It sounds like you might say more about this.

Rohan Rk
8:20 PM Oct 6
Resolve

+1 to Sherry's comment above. I think it'd be good to be explicit about this process. 'First ..., then ...' or some similar structure.

Rohan Rk
8:00 PM Oct 6
Resolve

Typo? 'develop'

Rohan Rk
8:08 PM Oct 6
Resolve

Good focus on impact. The wording's a bit awkward. I'd omit 'later' in this sentence.

Sherry Sarkar
10:26 AM Sep 20
Resolve

I feel uncertain what the purpose of this paragraph is. Is it the twofold response, or is it dendritic cells? How does the purpose of this paragraph relate the the next paragraphs?

apply targeted vaccinations. As the potential for clinical application becomes increasingly apparent, we must dive deeper into the pathophysiology of the tumor environment and subsequently question the relevance of these findings.

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Rohan Rk
8:24 PM Oct 6
Resolve
activate

Rohan Rk
8:27 PM Oct 6
Resolve
Why is this important?

Rohan Rk
8:28 PM Oct 6
**Delete:** *"a"*

Rohan Rk
8:32 PM Oct 6
**Add:** *"."*

Rohan Rk
8:28 PM Oct 6
**Add:** *"the"*

Sherry Sarkar
10:28 AM Sep 20
Resolve
Do you mean equipped?

Rohan Rk
8:32 PM Oct 6
**Replace:** *"preform"* with *"perform"*

Sherry Sarkar
10:29 AM Sep 20
Resolve
How do theses tests determined the "effictiveness of AuNP"? Can you summarize what positive / negative results might look like to prove / disprove the effectiveness?

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**Rohan Rk**
8:46 PM Oct 6
Resolve

I'm assuming you have or will have inline citations in your final draft. It's super useful in the lit review because it gives a bit more information about your sources without the reader having to delve into them.

**Rohan Rk**
8:42 PM Oct 6
Resolve

This paragraph seems to fit better in your introduction. Providing background information on gold nanoparticles and dendritic cells in the introduction would flow very nicely.

**Rohan Rk**
8:33 PM Oct 6

**Add:** *"."*

**Rohan Rk**
8:42 PM Oct 6

**Replace:** *"delivery"* with *"deliver"*

**Rohan Rk**
8:36 PM Oct 6

**Add:** *"."*

**Rohan Rk**
8:52 PM Oct 6
Resolve

Really good paragraph. It highlights the importance of your research while also clarifying aspects you mentioned in your introduction

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your introduction

Sherry Sarkar
10:32 AM Sep 20
Resolve

This might be dumb but I didn't realize NP stood for nanoparticle until just now XP.

Rohan Rk
8:29 PM Oct 6

**Delete space**

Rohan Rk
8:53 PM Oct 6

**Replace:** *“preformed”* with *“performed”*

Sherry Sarkar
10:34 AM Sep 20
Resolve

performed

Rohan Rk
8:37 PM Oct 6

**Add:** *“.”*

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Sherry Sarkar
10:34 AM Sep 20
Resolve
This is a long paragraph, not 100% sure what the main takeaway is. Maybe break it up into multiple paragraphs?

Rohan Rk
8:30 PM Oct 6
**Add space**

Rohan Rk
8:37 PM Oct 6
**Add:** "."

Rohan Rk
9:04 PM Oct 6
Resolve
+1 to Sherry's comment. This is a large wall of text. I'd break it up if you can.

## REFERENCES


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2. Ahmad, S., Zamry, A. A., Tan, H.-T. T., Wong, K. K., Lim, J., & Mohamud, R. (2017).

D. Madden's Comments:

Kalyn, your intro is really fantastic and is in good shape. Very rarely does this happen, but I don't have comments right now. We'll go over next steps in our meeting.

Amanda Grace Madden, Oct 23 at 9:07pm

ComLab:

Change the sentence structure of one sentence in the literature review. We stopped the sentence at the hyphen and broke it into two sentences.

Comments from my advisor:

Dr. Babensee commented a lot on my first proposal and has not made any comments sense then. There is no record of those comments, because I was not aware that we were supposed to save them. She talked very fast and we made the changes as she talked.

# Gold Nanoparticles Treatment in MCF-7 Spheroid Co-Cultures

Babensee Laboratory for Immunoengineering
Wallace H. Coulter Department of Biomedical Engineering
Georgia Institute of Technology

Kalyn Druhot
April 16, 2019

Faculty Member 1:
Dr. Julia Babensee

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Faculty Member 2:
Dr.

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# INTRODUCTION

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When the immune system responds to a threat in the body, it initiates a twofold response: innate and adaptive immune responses. Each respond simultaneously with different approaches to establish immunity in the body, but they ultimately work in partnership with each other to optimize the response pathway. While the innate response is immediate and nonspecific to the antigens of importance, the adaptive immune response takes several weeks to develop, but provides a tailored response to the antigens present. The adaptive immune response functions through T and B lymphocytes; allowing the body to retain memory in the form of a chemical structure copy of the antigen of importance. This memory allows the body to later elicit a quicker response to foreign invader upon subsequent exposure. Using a similar, antigen presenting mechanism, dendritic cells of our body are responsible for mediating both of these reaction pathways that comprise the immune system.[1]

AuNPs show high potential in the field of immunotherapies because they act as an adjuvant to evoke immuno-suppressive or immuno-activated response depending on their geometries and ligand coatings. The role of dendritic cells is to work as a gatekeeper to initiating immune response in our bodies as they are the most potent antigen presenting cell (APC). In

previous research, it has been shown that AuNPs can trigger the maturation of immature dendritic cells (iDCs) to activated or tolerogenic phenotypes.[2]

Researchers continue to fine tune nanoparticle technology for personalized immunotherapies and data indicates the efficacy of AuNP in eliciting anti-tumor response by providing a superior method to apply targeted vaccinations.[3,4] As the potential for clinical application becomes increasingly apparent, we must dive deeper into the pathophysiology of the tumor environment and subsequently question the relevance of these findings.

Dendritic cells are created and cultivated by differentiation in specific bone marrow cells called monocytes.[5] When an immune response occurs in the body, monocytes will cluster at the site of inflammation and systematically develop into immature dendritic cells (iDCs). Their major role in the innate and adaptive pathways is the activation of T cells.[6] Antigens are presented to immature dendritic cells, the iDCs phagocytose the antigens, and present the antigens on their surface. Once the antigens are presented on the surface of these cells the classification changes from immature to mature.[7]

The host environments' interaction with the tumor is one of the most important factors in cancer modeling.[8] In this study we investigate the effectiveness of AuNP application in the presence of tumor microenvironment equipped with dendritic cells, macrophages, and fibroblasts. We iteratively perform co-cultures of MCF-7 breast cancer cells with every combination of these cells in order to create a schematic which will assess applicability of AuNP use in breast cancer models. To make this assessment we will run tests to determine cell growth, viability, metastasis, and susceptibility to drugs. An indication of AuNPs working to combat cancer cells would be decreased cell growth, viability and metastasis and an increased susceptibility to drugs.

# LITERATURE REVIEW

In the last decade, nanoparticles have been a major area of focus for many researches as they show potential to carry and deliver drugs with very specific targeting, due to their low cell toxicity. Additionally, they show promise in the augmentation of the maturation of dendritic cells. Controlling the phenotype displayed in a mature dendritic cell allows for engineering and manipulation of the body's immune response.[9]

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3D extracellular matrices modulate intercellular interactions such as cell growth and metastasis.[12] Additionally, no tumor cell is purely one cell type. Fibroblast, dendritic cells, and monocytes are all part of the mix. Co-cultivation of these cell types into 3D spheroids produce protein concentrations analogous to tumors, while 2D models do not.[13,14]

The presence of these ECMs create a significant damper on AuNP delivery in vivo. A study preformed at the Indian Institute of Technology in Kanpur showed that the maximum achievable efficiency in vivo is only 2%. Although most of the NPs in this study were removed by Kupffer cells and other macrophages in the liver, NPs were also observed to pile up around

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# MATERIALS AND METHODS

This investigation is multifaceted in terms of experimental setup. First, the proper Matrigel concentration for the formation of MCF-7 spheroids must be confirmed. MCF-7 Cells will be plated at varying cell concentrations in mediums with varying composition of Matrigel to investigate which method most reliably produces spheroids. See the below plate layout for more information.

| | 1% Matrigel to equal parts colagen I | | | 3.5% Matrigel | | | 3.5% Matrigel to equal parts collagen I | | | control: just media | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | cell seed count |
| A | | | | | | | | | | | | | 5,000 |
| B | | | | | | | | | | | | | |
| C | | | | | | | | | | | | | 10,000 |
| D | | | | | | | | | | | | | |
| E | | | | | | | | | | | | | 15,000 |
| F | | | | | | | | | | | | | |
| G | | | | | | | | | | | | | |
| H | | | | | | | | | | | | | |

*Figure 1: Plate layout for determining optimal Matrigel concentration*

Cell viability will be assessed both optically (through the use of a microscope with the capability to take photographs and by using a Cell-Titer Glo 3D Cell Viability assay. The Cell-Titer Glo 3D Cell Viability assay will be performed as instructed by Promega, the distributor.

The next experiment to be performed will be adding AuNP treated dendritic cells to the MCF-7 cells in Matrigel to create a coculture of the two. Dendritic cells will be treated with AuNPs per the protocol perfected by another group of undergraduates in Dr. Babensee's lab. (their paper will eventually be linked here, but it has not been published yet). Subsequently,

macrophages will be added to the coculture and then fibroblasts native to human breast tissue. Assays will be conducted to determine cell growth, viability, metastasis, and susceptibility to drugs under these varying conditions.

# REFERENCES


1. Parkin, J. & Cohen, B. “An overview of the immune system.” The Lancet 357.9270 (2001): 1777-89. Web.
2. Ahmad, S., Zamry, A. A., Tan, H.-T. T., Wong, K. K., Lim, J., & Mohamud, R. (2017). Targeting dendritic cells through gold nanoparticles: A review on the cellular uptake and subsequent immunological properties. Molecular Immunology, 91, 123-133.
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Kalyn Druhot
April 16, 2019

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# TABLE OF CONTENTS

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# MATERIALS AND METHODS

This investigation is multifaceted in terms of experimental setup. Figure 1 is a schematic that breaks down the experiment into parts of a whole.

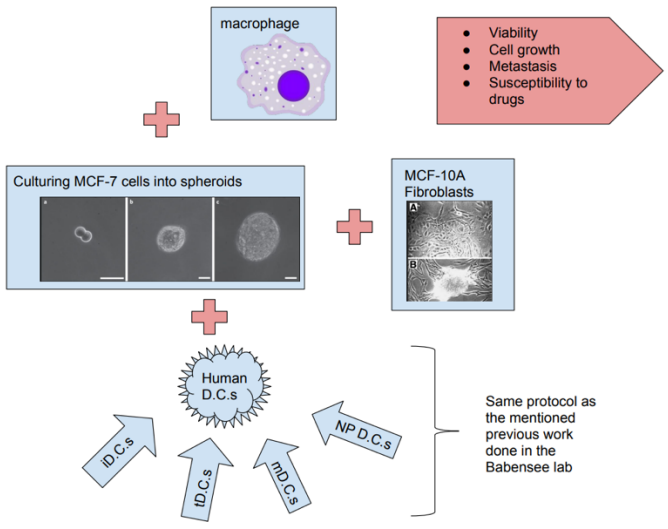



*Figure 1: Experimental Overview*

First, the proper Matrigel concentration for the formation of MCF-7 spheroids must be confirmed. MCF-7 Cells will be plated at varying cell concentrations in mediums with varying composition of Matrigel to investigate which method most reliably produces spheroids. While the stock solutions of Matrigel are made using a dilution series, preincubate 96-well microtiter plates to 37°C. The plates were precoated with 30 µL per well of the relevant matrix. The relevant matrix corresponds to the plate layout seen in Figure 2.

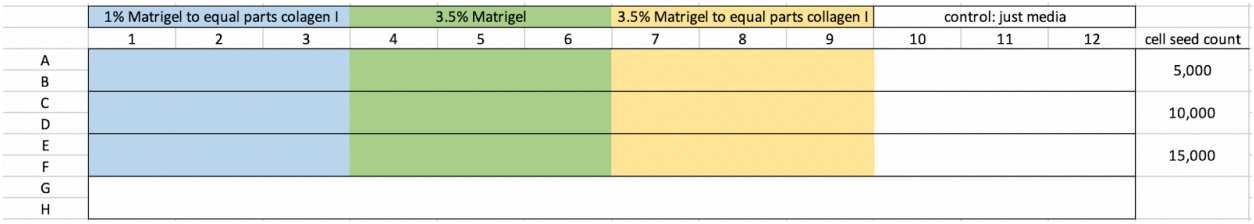



*Figure 2: Plate layout for determining optimal Matrigel concentration*

The MCF-7 cells were then passaged using Tripsin-EDTA and FBS. Next, cells were diluted to the double desired final concentrations as indicated in Figure 2. These discrete concentrations were then added to equal parts Matrigel to create the cell and Matrigel concentration indicated in the plate layout. Each well receives 60µL of this mixture. For example, to create the concentration in well A4 you will add 30µL of 7% Matrigel solution to 30µL of a solution that contains 10,000 MCF-7 cells. Cell viability will be assessed both optically (through the use of a microscope with the capability to take photographs) and by using a Cell-Titer Glo 3D Cell Viability assay. The Cell-Titer Glo 3D Cell Viability assay will be performed as instructed by Promega, the distributor.

The next experiment to be performed will be adding AuNP treated dendritic cells to the MCF-7 cells in Matrigel to create a coculture of the two. Dendritic cells will be treated with AuNPs per the protocol perfected by another group of undergraduates in Dr. Babensee's lab. (their paper will eventually be linked here, but it has not been published yet). These AuNP-

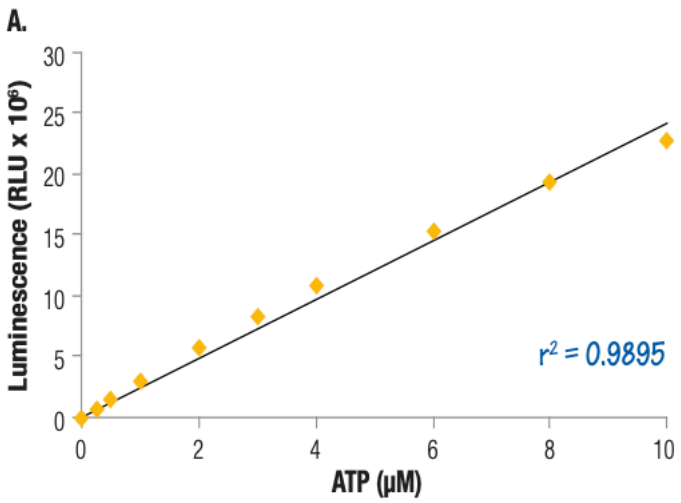
treated dendritic cells will be added to the culture of MCF-7 cells in a ratio of 2:1, a ratio used by previous studies.[21] As a control, untreated dendritic cells and MCF-7 cells will also be added together in a 2:1 ratio. Also both MCF-7 cells and dendritic cells will be seeded alone After this study, we will then study the effects of the use of the drug doxorubicin on both cocultures with AuNP-dendritic cells and cocultures with containing dendritic cells with no treatment.

Subsequently, macrophages will be added to the coculture and then fibroblasts will be added to the coculture that are native to human breast tissue. Assays will be conducted to determine cell growth, viability, metastasis, and susceptibility to drugs under these varying conditions.

## RESULTS (please note: all of this is hypothetical, we are still testing)

Data collected by the Celltiter Glo 3D assay is displayed bellow for each of the 9 testing groups.

An example of what figures 1-9 will look like:




ATP corresponds directly with cell count so these graphs can be processed to show cell count:

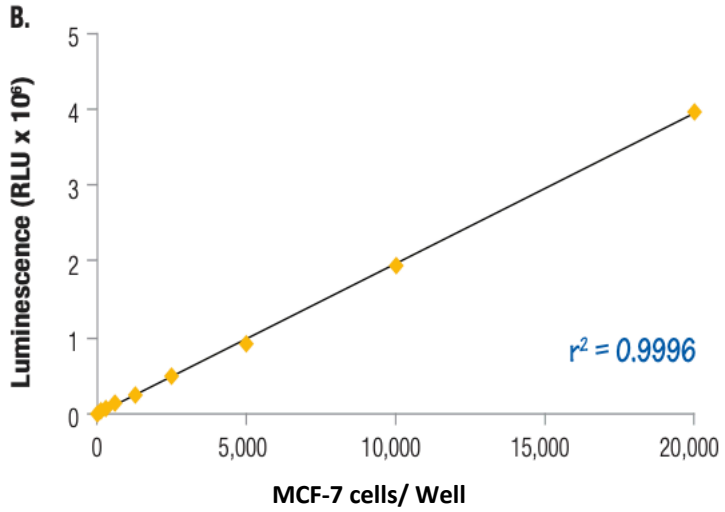
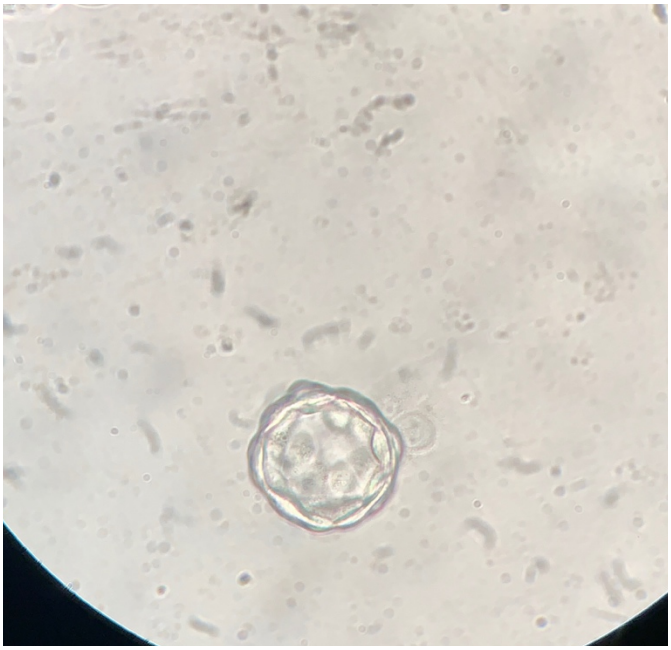



Photo images from a few of each category will be organized into a table:

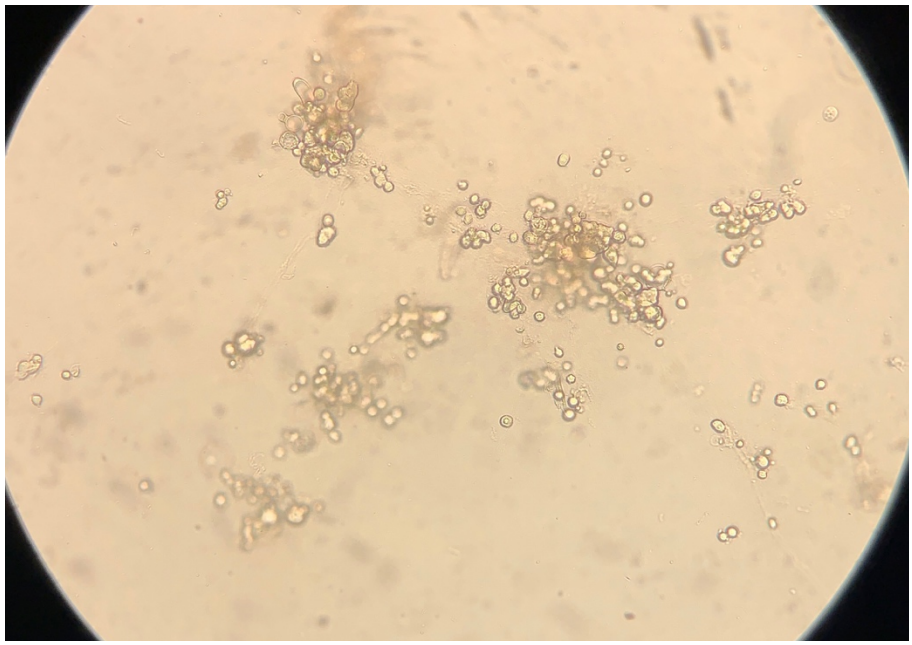
| | Matrigel/Collagen Concentrations |
|---|---|

| Cell Count | 1% Matrigel/1% Collagen | 3.5% Matrigel | 3.5% Matrigel/3.5% Collagen |
|---|---|---|---|
| 5,000 | (image) | (image) | (image) |
| 10,000 | (image) | (image) | (image) |
| 15,000 | (image) | (image) | (image) |

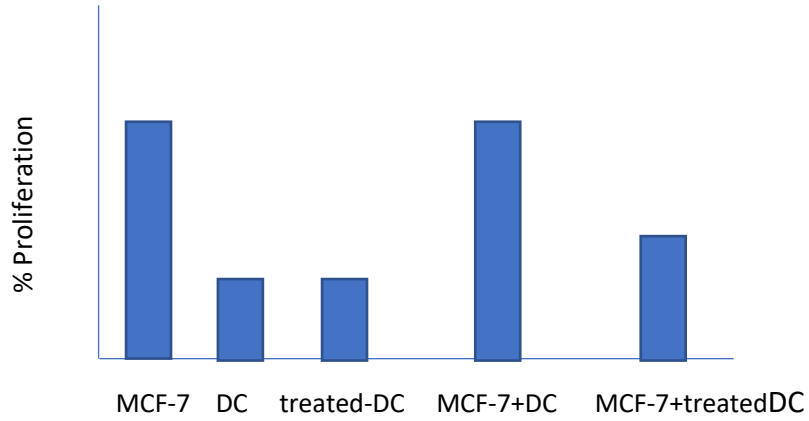
This is an example of one of the better spheroids



This is an example of one that did not aggregate as well



An example graph for the cocultures:




Sample tables for cocultures

Day1

| Day | MCF-7 | DC | treatedDC | MCF7+ DC | MCF-7 + treatedDC |
|---|---|---|---|---|---|
| 1 | Cell count | Cell count | Cell count | Cell count | Cell count |
| | (image) | (image) | (image) | (image) | (image) |
| 3 | Cell count | Cell count | Cell count | Cell count | Cell count |
| | (image) | (image) | (image) | (image) | (image) |
| 5 | Cell count | Cell count | Cell count | Cell count | Cell count |
| | (image) | (image) | (image) | (image) | (image) |

Similar tables will follow as fibroblasts and macrophages are added

*ANOVA statistical tests for significance of decrease here*

## DISSCUSION (please note: all of this is hypothetical, we are still testing)

Dendritic cells are integral players in the initiation of immune response in the body. Research has shown that the application of gold nanoparticles to dendritic cells has a direct impact on the phenotypic profiles seen in the DC differentiation process. This change suggests that gold nanoparticle-treated dendritic cells are better equip to fight cancer. The experiment we conducted put that suggestion to the test on a 3D tumor model. Our data indicates that 3D breast cancer spheroids that are grown in 3.5% Matrigel/3.5% collagen gels create the most consistent spheroids. Adding AuNP-treated dendritic cells to these 3D breast cancer spheroids yields promising results. We found a statistically significant decrease in cell viability and a decrease in

cell growth rates. *stats here* These were further decreased when the drug doxorubicin was applied to these spheroids. *stats here*

When fibroblasts are added to the coculture we found that it creates more consistent and symmetrical spheroids, also these spheroids responded to being cocultured with AuNP-treated dendritic cells more dramatically. *stats on cell viability and growth rate decreasing more that that of just MCF-7 + DC spheroids*

Although the addition of macrophages to the coculture also creates more consistent and symmetrical spheroids, these spheroids do not show as impressive of decreases in cell viability and growth rate compared to that of just MCF-7 + DC spheroids. *stats*. This indicates that cell-to-cell interaction between activated dendritic cells and macrophages might be counterproductive to the intended effects on the study. Macrophages are widely present near breast cancer tumors so this could prove to be a major obstacle. Further research should be done to confirm.

## CONCLUSION (please note: all of this is hypothetical, we are still testing)

AuNP-treated dendritic cells have a significant impact on the slowing of MCF-7 breast cancer spheroids growth and viability. The addition of fibroblasts and macrophages to this coculture make it a more accurate model of real breast cancer tumors that are found in cancer patients. The addition of these cells to the spheroids is integral to an honest evaluation of the effectiveness of using AuNP-treated dendritic cells for cancer therapy. Our data indicates that the addition of fibroblasts to the coculture makes the treatment more effective, while the addition of macrophages to the coculture makes the treatment less effective. This should be explored further in a mouse model to determine if the presence macrophages will make this type of treatment ineffective.

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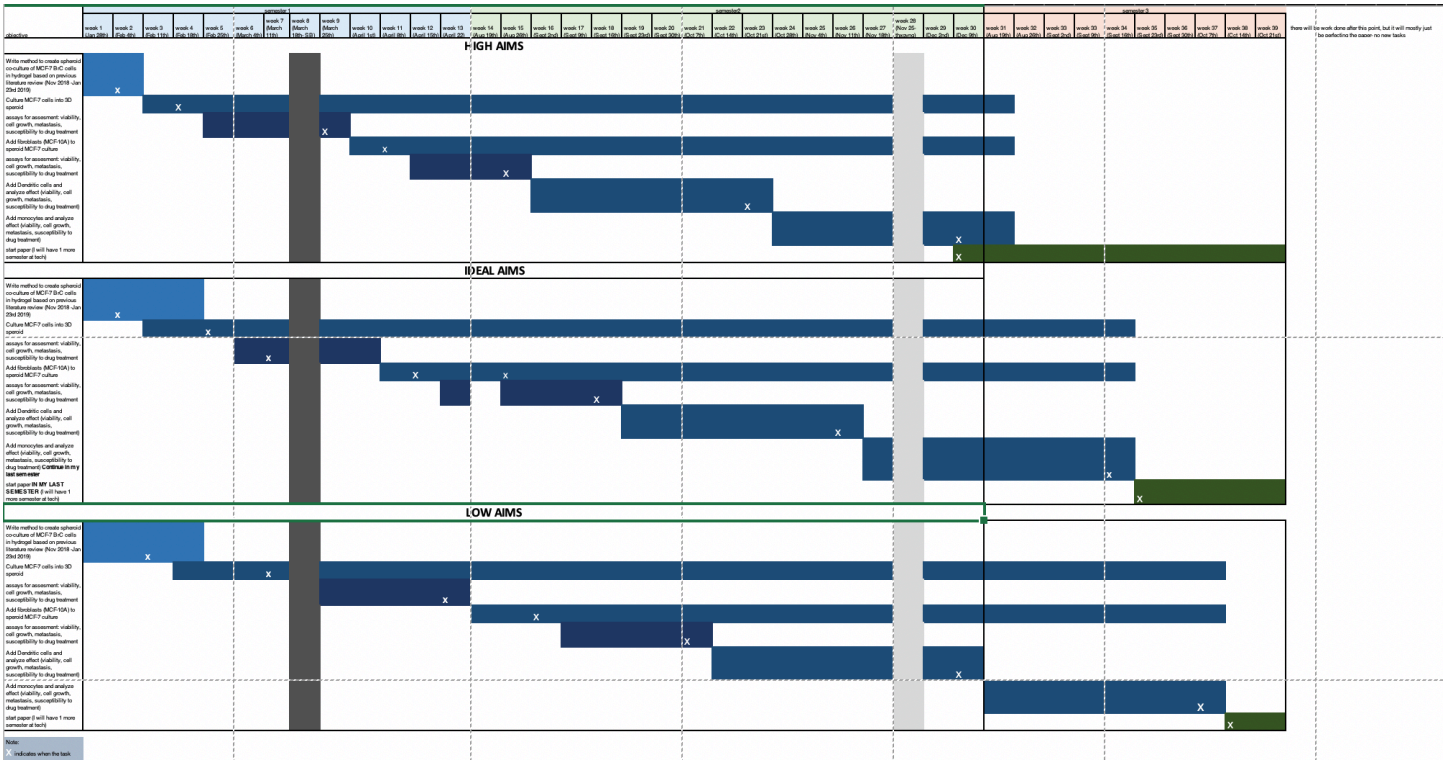
objective
semester 1
semester2
semester 3
week 1 (Jan 28th)
week 2 (Feb 4th)
week 3 (Feb 11th)
week 4 (Feb 18th)
week 5 (Feb 25th)
week 6 (March 4th)
week 7 (March 11th)
week 8 (March 18th, 21st)
week 9 (March 25th)
week 10 (April 1st)
week 11 (April 8th)
week 12 (April 15th)
week 13 (April 22nd)
week 14 (Aug 19th)
week 15 (Aug 26th)
week 16 (Sept 2nd)
week 17 (Sept 9th)
week 18 (Sept 16th)
week 19 (Sept 23rd)
week 20 (Sept 30th)
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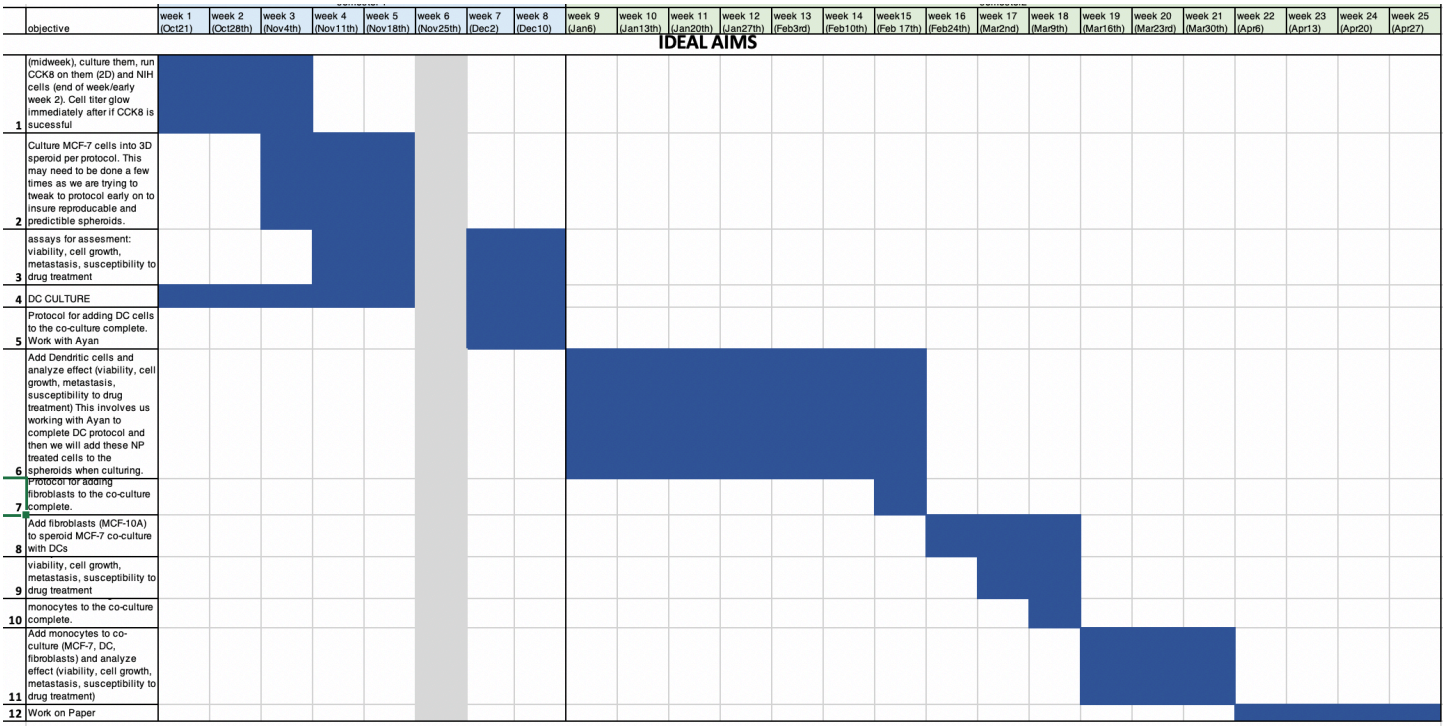
week 31 (Aug 12th)
week 32 (Aug 30th)
week 33 (Sept 2nd)
week 33 (Sept 9th)
week 34 (Sept 16th)
week 35 (Sept 23rd)
week 36 (Sept 30th)
week 37 (Oct 7th)
week 38 (Oct 14th)
week 39 (Oct 21st)
there will be work done after this point, but it will mostly just be perfecting the paper- no new tasks
HIGH AIMS
Write method to create spheroid co-culture of MCF7 BrC cells in hydrogel based on previous literature review (Nov 2018 -Jan 23rd 2019)
Culture MCF-7 cells into 3D speroid
assays for assesment: viability, cell growth, metastasis, susceptibility to drug treatment
Add fibroblasts (MCF-10A) to speroid MCF-7 culture
assays for assesment: viability, cell growth, metastasis, susceptibility to drug treatment
Add Dendritic cells and analyze effect (viability, cell growth, metastasis, susceptibility to drug treatment)
Add monocytes and analyze effect (viability, cell growth, metastasis, susceptibility to drug treatment)
start paper (I will have 1 more semester at tech)
IDEAL AIMS
Write method to create spheroid co-culture of MCF7 BrC cells in hydrogel based on previous literature review (Nov 2018 -Jan 23rd 2019)
Culture MCF-7 cells into 3D speroid
assays for assesment: viability, cell growth, metastasis, susceptibility to drug treatment
Add fibroblasts (MCF-10A) to speroid MCF-7 culture
assays for assesment: viability, cell growth, metastasis, susceptibility to drug treatment
Add Dendritic cells and analyze effect (viability, cell growth, metastasis, susceptibility to drug treatment)
Add monocytes and analyze effect (viability, cell growth, metastasis, susceptibility to drug treatment) Continue in my last semester
start paper IN MY LAST SEMESTER (I will have 1 more semester at tech)
LOW AIMS
Write method to create spheroid co-culture of MCF7 BrC cells in hydrogel based on previous literature review (Nov 2018 -Jan 23rd 2019)
Culture MCF-7 cells into 3D speroid
assays for assesment: viability, cell growth, metastasis, susceptibility to drug treatment
Add fibroblasts (MCF-10A) to speroid MCF-7 culture
assays for assesment: viability, cell growth, metastasis, susceptibility to drug treatment
Add Dendritic cells and analyze effect (viability, cell growth, metastasis, susceptibility to drug treatment)
Add monocytes and analyze effect (viability, cell growth, metastasis, susceptibility to drug treatment)
start paper (I will have 1 more semester at tech)
Note:
'x' indicates when the task


objective
week 1 (Oct21)
week 2 (Oct28th)
week 3 (Nov4th)
week 4 (Nov11th)
week 5 (Nov18th)
week 6 (Nov25th)
week 7 (Dec2)
week 8 (Dec10)
week 9 (Jan6)
week 10 (Jan13th)
week 11 (Jan20th)
week 12 (Jan27th)
week 13 (Feb3rd)
week 14 (Feb10th)
week15 (Feb 17th)
week 16 (Feb24th)
week 17 (Mar2nd)
week 18 (Mar9th)
week 19 (Mar16th)
week 20 (Mar23rd)
week 21 (Mar30th)
week 22 (Apr6)
week 23 (Apr13)
week 24 (Apr20)
week 25 (Apr27)
IDEAL AIMS
1 (midweek), culture them, run CCK8 on them (2D) and NIH cells (end of week/early week 2). Cell titer glow immediately after if CCK8 is sucessful
2 Culture MCF-7 cells into 3D speroid per protocol. This may need to be done a few times as we are trying to tweak to protocol early on to insure reproducable and predictible spheroids.
3 assays for assesment: viability, cell growth, metastasis, susceptibility to drug treatment
4 DC CULTURE
5 Protocol for adding DC cells to the co-culture complete. Work with Ayan
6 Add Dendritic cells and analyze effect (viability, cell growth, metastasis, susceptibility to drug treatment) This involves us working with Ayan to complete DC protocol and then we will add these NP treated cells to the spheroids when culturing.
7 Protocol for adding fibroblasts to the co-culture complete.
8 Add fibroblasts (MCF-10A) to speroid MCF-7 co-culture with DCs
9 viability, cell growth, metastasis, susceptibility to drug treatment
10 monocytes to the co-culture complete.
11 Add monocytes to co-culture (MCF-7, DC, fibroblasts) and analyze effect (viability, cell growth, metastasis, susceptibility to drug treatment)
12 Work on Paper